DETECTION OF *CAMPYLOBACTER* FROM BROILER CARCASS RINSE SAMPLES UTILIZING THE TECRA VISUAL IMMUNOASSAY (VIA)

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ABSTRACT

Poultry meat is considered to be a major vector of transmission of Campylobacter, *either directly, through consumption of poorly prepared product or cross-contamination, or indirectly, through introduction of the bacterium into the food production environment. Efficient detection of* Campylobacter *is intrinsic to the management of the pathogen during poultry production. The TECRA* Campylobacter *Visual Immunoassay (CAMVIA) protocol, enrichment in a proprietary TECRA* Campylobacter *enrichment broth followed by an ELISA, was compared with a conventional cultural method, with enrichment in Bolton medium (containing lysed horse blood), followed by plating onto Campy-cefex agar. Of the 398 broiler carcass rinses tested from 19 commercial processing plants, a total of 350 carcasses (88%) were found to be positive for* Campylobacter *by at least one method. The TECRA CAMVIA yielded 317* Campylobacter*-positive results, four of which were false positive and 22 of which were false negative, while conventional enrichment and plating detected the bacterium in only 280 samples with 48 false negatives. These data demonstrate that the TECRA CAMVIA has a*

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statistically higher sensitivity and specificity than a conventional culture procedure using Bolton broth as the enrichment.

PRACTICAL APPLICATIONS

Standard *Campylobacter* methodology procedures currently take 4–5 days for detection of *Campylobacter* spp. in a sample when using an enrichment step. The TECRA *Campylobacter* Visual Immunoassay (VIA) reduces the amount of time it takes to determine whether *Campylobacter* spp. are present on poultry carcasses that have been rinsed. The procedure can effectively determine the presence of *Campylobacter* spp. in 2 days and has a statistically higher sensitivity and specificity than a conventional culture procedure using Bolton broth as the enrichment.

INTRODUCTION

Approximately 76 million cases of food-borne illnesses occur annually in the United States (Mead *et al.* 1999). *Campylobacter* is a major cause of acute bacterial gastroenteritis in the United States, accounting for approximately 2.4 million cases of illness per year (Mead *et al.* 1999). Campylobacteriosis has been closely associated with the consumption of undercooked commercial poultry products and cross-contamination from raw poultry products. Seventy-one percent of poultry carcasses and 91% of the retail chicken products were shown to be contaminated with *Campylobacter* (Zhao *et al.* 2001). Conventional cultural methods for detecting *Campylobacter* involve enrichment in selective broths, followed by isolation of the organism on selective agar (Endtz *et al.* 1991). *Campylobacter* have demanding growth requirements (microaerobic conditions) and traditional cultural methods for recovery and isolation are laborious and time-consuming, and can take from 4 to 5 days to complete (Cox *et al.* 2007).

In recent years, numerous studies have been conducted to develop or evaluate rapid methods for the detection of *Campylobacter* spp. from poultry samples (Cloak *et al.* 2001; Wicker *et al.* 2001; Hong *et al.* 2003). Recently developed quicker methods for the detection of *Campylobacter* from foods have utilized alternative methods such as antibody- (Tsai *et al.* 1994) or DNA-based assays (Hong *et al.* 2003). Two-step ELISA-based methods that incorporate improved enrichment broths and antibody-based ELISAs are being developed where results are obtained within 2 days. A recent study evaluated a Transia Plate *Campylobacter* method, a two-step process in which a sample is enriched in Bolton broth for 48 h followed by a short ELISA test (Wicker

et al. 2001). In that study, the method was found to be as sensitive and specific as the ISO reference method utilized for detecting *Campylobacter* in food. The presumptive positive samples and negative samples were determined within 2 days, whereas the ISO method required 4 days. In recent years, a similar two-step process, TECRA *Campylobacter* Visual Immunoassay (CAMVIA) has been developed. In this procedure, a propriety broth is utilized, which is incubated in aerobic conditions over 2 days, and then a visual immunoassay is performed where results can be obtained in a few hours after enrichment. The objective of this study was to compare the TECRA CAMVIA to a standard conventional culture method incorporating Bolton broth for recovery of *Campylobacter* spp. from carcass rinse samples.

MATERIALS AND METHODS

Experimental Design

Carcasses (398) were obtained at the rehang and post-chill stages of processing from 19 different commercial poultry processing plants throughout the continental U.S.A. Each carcass was put into a rinse bag and 100 mL of deionized water was added, and the carcass was rinsed for 1 min. Samples were then packed on ice and transported back to the laboratory for evaluation. From each sample, a 10-mL aliquot was transferred to 90 mL of both Bolton enrichment broth (containing 5% lysed horse blood) and TECRA enrichment broth and standard laboratory procedures for each method performed for the recovery of *Campylobacter* spp. (Cox *et al.* 2007).

A U.S. Department of Agriculture Standard Cultural Procedure

Briefly, Bolton enrichment broth (containing 5% lysed horse blood) was used as the selective enrichment and incubated in a microaerophilic atmosphere at 42C for 48 h. A 0.1-mL solution of the selective enrichment broth from each sample was then streaked onto *Campy*-cefex agar plates and incubated in a microaerophilic atmosphere at 42C for 48 h. Following incubation, plates were observed for presumptive *Campylobacter* colonies. Presumptive colonies were confirmed by microscopic observation of characteristic spiral cells and tumbling motility in wet mount preparations and further confirmed through latex agglutination (Microgen Bioproducts, Surrey, UK).

TECRA CAMVIA Procedure

A proprietary TECRA selective enrichment broth was incubated at 42C for 48 h in an aerobic atmosphere. The immunoassay uses breakable well

strips coated with antibodies directed against specific *Campylobacter* antigens. All the reagents were supplied in a ready-to-use format. The presence of *Campylobacter* was indicated when the bound conjugate converts the substrate to a green/blue color as per manufacturer's instructions.

TECRA Enrichment Broth Cultural Procedure

A 0.1 mL solution of the selective enrichment broth from each sample was streaked onto *Campy*-cefex agar plates and incubated in a microaerophilic atmosphere at 42C for 48 h. Following incubation, above-standard laboratory procedures were performed for confirmation.

Statistical Analysis

Statistically significant differences between procedures were determined by Fisher's Exact Test (GrahPad Software, Inc., San Diego, CA) within a 95% confidence interval.

RESULTS AND DISCUSSION

Of the 398 broiler carcass rinses, from 19 commercial processing plants tested, a total of 350 (88%) were positive for *Campylobacter* as confirmed from the combination of the TECRA broth and Bolton broth cultural procedures where aliquots were plated onto Campy-cefex agar and confirmed through wet mounts and latex agglutination (Fig. 1). The incidence of *Campylobacter* in this study on chicken carcasses by the combination of the two enrichment procedures was similar to results found in other studies (Harrison *et al.* 2001; Zhao *et al.* 2001; Oyarzabal *et al.* 2005; Scherer *et al.* 2006). A recent study, Scherer *et al.* (2006), found that 77% of carcass rinse samples were positive for *Campylobacter*. In another study, only 26% of the carcass rinse samples were positive for *Campylobacter* (Stern and Pretanik 2006); however, no enrichment procedure was utilized and only direct plating was performed where the sensitivity level was reported as $\log_{10}$ 3.6 per sample. This could explain the considerably lower level of detection compared with the present study, especially if stressed or injured cells were present in the samples that were tested by the researchers.

Significantly more *Campylobacter* ($P = 0.0032$) were found using the TECRA CAMVIA procedure (313/398) compared with the standard procedure of enriching in Bolton broth and then plating (280/398) for the recovery of *Campylobacter* from the carcass rinses (Fig. 1). A highly significant difference ($P < 0.0001$) in recovery of *Campylobacter* was also observed between the plating of the TECRA (328/398) and Bolton broth (280/398) onto Campy-

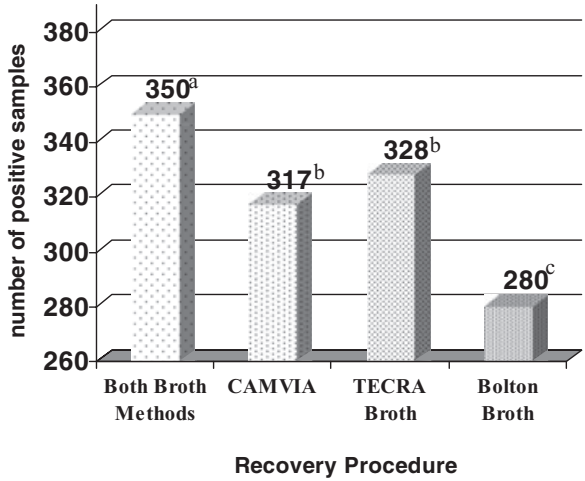

FIG. 1. COMPARISON OF TECRA CAMVIA, TECRA BROTH AND BOLTON BROTH PROCEDURES FOR THE RECOVERY OF *CAMPYLOBACTER* FROM A TOTAL OF 398 CARCASS RINSE SAMPLES
Significance of incidence by method as indicated by different lower case letters ($P < 0.05$).

cefex agar plates (Fig. 1). However, no significant difference ($P > 0.05$) was noted between the TECRA CAMVIA (317/398) procedure and the TECRA broth (328/398) plating procedure (Fig. 1). Using the TECRA CAMVIA procedure, *Campylobacter* was recovered from 79.6% of the samples. Using the U.S. Department of Agriculture (USDA) Bolton broth procedure, *Campylobacter* was recovered from 70% of the samples. Plating onto Campy-cefex agar from the TECRA broth recovered *Campylobacter* from 82% of the samples.

Overall, the TECRA CAMVIA procedure provided results within a 2-day period and was found to have greater sensitivity than a current USDA method utilizing Bolton broth for recovery of *Campylobacter*. By comparing the Bolton broth to the TECRA broth, the increased sensitivity of the TECRA CAMVIA procedure was attributable to the proprietary broth that is utilized for the procedure. However, false negatives were found in both procedures. From subjective observation of the Campy-cefex plates, the TECRA broth appeared to be more effective in preventing growth of background microflora than the Bolton broth that probably contributed to overall increased sensitivity (data not shown). The 48 false negative results from the USDA method were likely attributed to suppression of *Campylobacter* in the Bolton enrichment broth, due primarily to overgrowth by the background microflora, which made it difficult to identify *Campylobacter* bacteria-like colonies. Of the negative

TECRA CAMVIA and TECRA broth results, 15 were shown to be false negative by comparison to the Bolton broth method, and of those, 10 came from one plant. All these false negatives came from rinses of carcasses collected toward the end of the production process, suggesting that severely injured *Campylobacter* may not recover well in the TECRA enrichment broth, though very low numbers were detected from similar samples from other plants. The decreased sensitivity of recovery may have been because of the differences in antimicrobials that each broth's supplements contain. The antibiotic supplement for Bolton's broth contains cefoperazone, vancomycin, trimethoprim and cycloheximide. The antibiotic supplement for TECRA broth contains trimethoprim, rifampicin and polymyxin B.

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